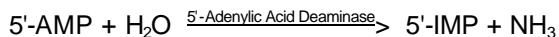


**Enzymatic Assay of 5'-ADENYLIC ACID DEAMINASE
(EC 3.5.4.6)**

PRINCIPLE:



Abbreviations used:

5'-AMP = Adenosine 5'-Monophosphate

5'-IMP = Inosine 5'-Monophosphate

CONDITIONS: T = 25°C, pH = 6.5, $A_{265\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 10 mM Sodium Citrate Buffer, pH 6.5 at 25°C.
(Prepare 1 liter in deionized water using Sodium Citrate, Trisodium Salt, Dihydrate, Sigma Prod. No. S-4641. Adjust to pH 6.5 at 25°C with 1 M HCl.)
- B. 0.04 mM Adenosine 5'-Monophosphate Solution (5'-AMP)¹
(Prepare 50 ml in Reagent A using Adenosine 5'-Monophosphate, Sodium Salt, Sigma Prod. No. A-1877. **PREPARE FRESH.**)
- C. 1 M Potassium Chloride Solution (KCl)
(Prepare 50 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-3911. **PREPARE FRESH.**)
- D. 5'-Adenylic Acid Deaminase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 0.4 unit/ml of 5'-Adenylic Acid Deaminase in cold Reagent C.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable quartz cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent B (5'-AMP)	2.90	2.90

Equilibrate to 25°C. Monitor the $A_{265\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C (KCl)	-----	0.10
Reagent D (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the decrease in $A_{265\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{265\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{265\text{nm}}/\text{min Test} - A_{265\text{nm}}/\text{min Blank})(3)(\text{df})}{(8.1)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

8.1 = Millimolar extinction coefficient of 5'-AMP at 265 nm

0.1 = Volume (in milliliters) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will deaminate 1.0 μmole of 5'-AMP to 5'-IMP per minute at pH 6.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 9.7 mM sodium citrate, 0.04 mM adenosine 5'-monophosphate, 33 mM potassium chloride and 0.02 - 0.04 unit 5'-adenylic acid deaminase.

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(EC 3.5.4.6)**

REFERENCE:

Murphy, J., Baker, D.C., Behling, C., and Turner, R.A., (1982) *Analytical Biochemistry* **122**, 328-337

NOTES:

1. The substrate concentration should not be significantly increased above that listed in the procedure. Higher concentrations result in a deviation from Beers Law leading to errors in the rate determination according to Murphy, J. et al. (1982).
2. This assay is based on the cited reference.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.